JSR Publications

Available Online

J. Sci. Res. **4** (1), 183-192 (2012)

JOURNAL OF SCIENTIFIC RESEARCH

www.banglajol.info/index.php/JSR

Synthesis, Antitumor and Antimicrobial Activities of Some Novel 1-(Substituted)-3-Methyl-1*H*-Pyrazol-5(4*H*)-One

R. V. Antre[1*], A. Cendilkumar[2], R. Nagarajan[3], D. Goli[2], and R. J. Oswal[1]

[1]Medicinal Chemistry Research Laboratory, JSPM`S Charak College of Pharmacy and Research, Wagholi, Pune, Maharashtra 412207, India

[2]Medicinal Chemistry Research Laboratory, Acharya and B.M. Reddy College of Pharmacy, Bangalore, Karnataka 560090, India

[3]Organic Chemistry Research Laboratory, School of Chemistry, University of Hyderabad, Central University (P.O.), Hyderabad, Andhrapradesh 500046, India

Received 8 February 2011, accepted in final revised form 20 October 2011

Abstract

Several 1-(substituted)-3-methyl-1H-pyrazol-5(4H)-one as **RA1-RA9** were synthesized and compounds were screened for antitumor activity against Ehrlich ascites carcinoma (EAC) cells and antimicrobial activity. Elemental analysis, mass spectral data, ^{1}H-NMR, and IR confirmed the structure of the newly synthesized compounds. Some of the tested compounds showed good antitumor and antimicrobial activity. Compounds **RA1, RA4,** and **RA9** exhibit highest antitumor activity against EAC cells in comparison with 5-flurouracil as standard drug.

Keywords: Ehrlich ascites carcinoma; Pyrazolone; Antitumor; N-substitution; Antimicrobial.

doi:10.3329/jsr.v4i1.7027 J. Sci. Res. **4** (1), 183-192 (2012)

1. Introduction

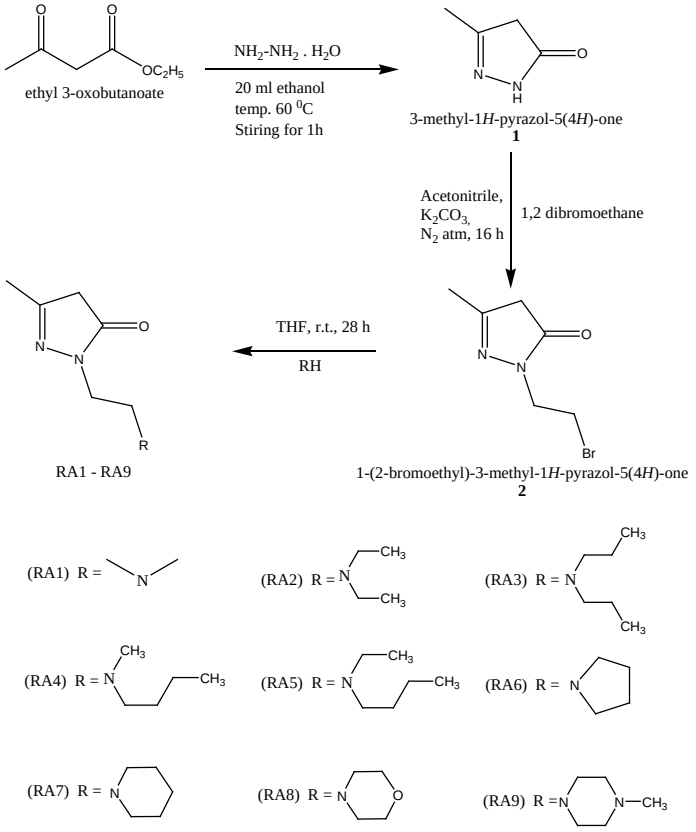
Pyrazolone ring system has been consistently rewarded as a promising molecule because of its broad spectrum pharmacological activities like antitubercular [1], antimicrobial [2], anticancer [3,4], antiviral [5], analgesic [6-9], anti-inflammatory [8, 9], antipyretic [9, 10], ulcerogenic [11], lipid peroxidation [11]. Structure activity relationship studies of pyrazolone ring system revealed in various literatures [12-14], suggest position N-1, C-3, C-4 are very much important for structure activity studies and C-3 should be attached to different heterocyclic rings for better chemotherapeutic activity. Since N-substitutions in pyrazolone exhibit biologically active compounds [15], we were interested in preparing compounds containing them. In view of these observations, we report herein the reaction of 3-methyl-1*H*-pyrazol-5(4*H*)-one (**1**) and 1,2-dibromo ethane in acetonitrile with

* *Corresponding author*: rishiantre@gmail.com

presence of K_2CO_3 forms 1-(2-bromoethyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one (**2**). Compound **2** further reacted with substituted amines gives formation of compound **RA1-RA9.** The compound **RA1-RA9** screened for their antitumor as well as antimicrobial activities.

2. Chemistry

In scheme-1 ethyl acetoacetate was reacted with hydrazine hydrate in presence of ethanol forms 3-methyl-1*H*-pyrazol-5(4*H*)-one (**1**). The treatment of **1** with 1,2-dibromo ethane in acetonitrile with presence of K_2CO_3 forms 1-(2-bromoethyl)-3-methyl-1*H*-pyrazol-5(4*H*)-one **2**. This **2** reacts with substituted amines forms **RA1-RA9**. The ^{1}H-NMR spectra of 3-methyl-1*H*-pyrazol-5(4*H*)-one (**1)** showed broad peak of -NH at δ 10.5 ppm two protons and presence of broad peak at δ 9.086 ppm due to one proton established that N-substitution occurs in product (**2)**. The ^{1}H-NMR, MS, IR and elemental analysis supported the structure of title compounds.



Scheme 1. Synthesis of compounds RA1-RA9.

3. Experimental

Analytical TLC was performed on Silica gel F254 plates (Merck) with visualization by UV light. All the products obtained were purified by column chromatography using silica gel (100-200 mesh). Hexane was used as a co-eluent. ^{1}H NMR was recorded in Brucker 400 MHz spectrometer. LC–MS was used for the mass spectral analysis. IR spectra were recorded on a FT-IR spectrometer using KBr pellets. Elemental analysis was carried out in CHN analyzer EA-1112, Thermo Finnigan. All spectral studies were carried out at elemental analysis laboratory in the School of Chemistry, University of Hyderabad, India. Melting points were determined in open capillaries on a Thermonik melting point apparatus, Mumbai, India and are uncorrected.

Synthesis of 3-methyl-1*H*-pyrazol-5(4*H*)-one (1): This compound was synthesized by methods reported earlier [20]. Ethyl acetoacetate (0.1 mole) was taken in conical flask and hydrazine hydrate (0.2 mole) in ethanol (20 ml) was added drop wise to it with stirring. The temperature rose during this addition and it was maintained at 60 °C when a crystalline solid was formed. The reaction-mixture was further stirred for 1 h at room temperature, then cooled in an ice bath to complete the crystallization. Separated solid was washed with ice cold ethanol.

3-methyl-1*H*-pyrazol-5(4*H*)-one (1): Yield 82%; mp 222-225 °C; IR (KBr) *v* cm^{-1}: 2997, 1651, 1551, 1502, 1453. ^{1}H-NMR (DMSO-*d6*) δ: 10.50 (s, -NH, 2H), 5.20 (s, -CH, 1H), 2.07 (s, -CH_3, 3H). MS *m/z*: 99 (M+1). *Anal.* Calcd for $C_4H_6N_2O$: C, 48.97; H, 6.16; N, 28.56. Found: C, 48.91; H, 6.84; N, 28.43.

Synthesis of 1-(2-bromoethyl)-3-methyl-1H-pyrazol-5(4H)-one (2): 3-methyl-1*H*-pyrazol-5(4*H*)-one **(1)** (0.1 mol) in 20 mL acetonitrile was added with 4 gm of K_2CO_3. The mixture was stirred for 10 minutes under nitrogen atmosphere. 1,2-dibromo ethane (0.015 mol) was added to this mixture which was stirred at room temperature for 16 h under nitrogen atmosphere. The completion of the reaction was monitored on silica gel 60 F254 precoated TLC plates (Merck) with visualization by UV light. After completion of reaction acetonitrile was removed under reduced pressure. The precipitate was collected; water and ethyl acetate (1:1) 50 mL were added to solid product. By using separating funnel organic layer was collected. Ethyl acetate was removed under reduced pressure and product collected. Further desired product was obtained by column chromatography and characterized by spectral studies.

1-(2-bromoethyl)-3-methyl-1H-pyrazol-5(4H)-one (2): Yield 53%; mp 87-89 °C; IR (KBr) *v* cm^{-1}: 2997, 1651, 1551, 1501, 1218, 661. ^{1}H-NMR (DMSO-*d6*) δ: 9.08 (s, -NH, 1H), 5.50 (s, -CH, 1H), 4.42 (t, -CH_2, 2H), 3.63 (t, -CH_2, 2H), 2.23 (s, -CH_3, 3H). MS *m/z*: 207, 125. *Anal.* Calcd for $C_6H_9BrN_2O$: C, 35.14; H, 4.42; N, 13.66. Found: C, 35.21; H, 4.28; N, 13.32.

Synthesis of 1-(2-(dimethylamino)ethyl)-3-methyl-1H-pyrazol-5(4H)-one (RA1): 1-(2-bromoethyl)3-methyl-1*H*-pyrazol-5(4*H*)-one (**2**) of 0.002 mol and N,N dimethylamine 0.004 mol were mixed in a THF, and stirred at room temperature for 28 h. THF was removed under reduced pressure. The product was collected and the desired product was isolated by column chromatography. The product was then characterized by spectral analysis. The compounds RA2-RA10 were obtained in a similar manner.

1-(2-(dimethylamino)ethyl)-3-methyl-1H-pyrazol-5(4H)-one (RA1): Yield 65%; mp 103-105 °C; IR (KBr) *v* cm^{-1}: 2995, 1691, 1512, 1501, 1251. ^{1}H-NMR (DMSO-*d6*) δ: 9.08 (s, -NH, 1H), 5.50 (s, -CH, 1H), 4.42 (t, -CH_2, 2H), 3.63 (t, -CH_2, 2H), 2.23 (s, CH_3, 3H), 2.35 (s, CH_3, 6H). MS *m/z*: 170 (M+1). *Anal.* Calcd for $C_8H_{15}N_3O$: C, 56.78; H, 8.93; N, 24.83. Found: C, 56.54; H, 8.80; N, 24.33.

1-(2-(diethylamino)ethyl)-3-methyl-1H-pyrazol-5(4H)-one (RA2): Yield 62%; mp 132-134 °C; IR (KBr) *v* cm^{-1}: 2999, 1694, 1523, 1501, 1254. ^{1}H-NMR (DMSO-*d6*) δ: 9.08 (s, -NH, 1H), 5.50 (s, -CH, 1H), 4.42 (t, -CH_2, 2H), 3.63 (t, -CH_2, 2H), 3.53 (s, -CH_3, 6H), 3.13 (s, -CH_3, 3H), 2.45 (m, -CH_2, 2H), 2.39 (m, -CH_2, 2H). MS *m/z*: 198 (M+1). *Anal.* Calcd for $C_{10}H_{19}N_3O$: C, 60.88; H, 9.71; N, 21.30. Found: C, 60.44; H, 9.78; N, 21.13.

1-(2-(diisopropylamino)ethyl)-3-methyl-1H-pyrazol-5(4H)-one (RA3): Yield 69%; mp 100-103 °C; IR (KBr) *v* cm^{-1}: 2997, 1697, 1514, 1258. ^{1}H-NMR (DMSO-*d6*) δ: 9.08 (s, -NH, 1H), 5.50 (s, -CH, 1H), 4.42 (t, -CH_2, 2H), 3.63 (t, -CH_2, 2H), 3.34 (d, -CH_3, 12H), 2.88 (m, -CH_2, 2H), 2.23 (s, -CH_3, 3H). MS *m/z*: 226 (M+1). *Anal.* Calcd for $C_{12}H_{23}N_3O$: C, 63.96; H, 10.29; N, 18.65. Found: C, 63.14; H, 10.87; N, 18.23.

1-(2-(butyl(methyl)amino)ethyl)-3-methyl-1H-pyrazol-5(4H)-one (RA4): Yield 72%; mp 110-112 °C; IR (KBr) *v* cm^{-1}: 2998, 1692, 1523, 1509, 1260. ^{1}H-NMR (DMSO-*d6*) δ: 9.08 (s, -NH, 1H), 5.50 (s, -CH, 1H), 4.42 (t, -CH_2, 2H), 3.92 (t, -CH_3, 3H), 3.63 (t, -CH_2, 2H), 2.83 (t, -CH_2, 1H), 2.40 (m, -CH_2, 1H), 2.23 (s, -CH_3, 3H), 2.31 (m, -CH_2, 1H), 2.12 (s, -CH_3, 1H). MS *m/z*: 212 (M+1). *Anal.* Calcd for $C_{11}H_{21}N_3O$: C, 62.52; H, 10.02; N, 19.89. Found: C, 62.42; H, 10.07; N, 19.20.

1-(2-(butyl(ethyl)amino)ethyl)-3-methyl-1H-pyrazol-5(4H)-one (RA5): Yield 65%; mp 123-125 °C; IR (KBr) *v* cm^{-1}: 2987, 1691, 1501, 1268. ^{1}H-NMR (DMSO-*d6*) δ: 9.08 (s, -NH, 1H), 5.50 (s, -CH, 1H), 4.42 (t, -CH_2, 2H), 3.82 (t, -CH_3, 6H), 3.63 (t, -CH_2, 2H), 2.83 (t, -CH_2, 1H), 2.40 (m, -CH_2, 1H), 2.31 (m, -CH_2, 1H), 2.23 (s, -CH_3, 3H), 2.12 (s, -CH_3, 1H). MS *m/z*: 226 (M+1). *Anal.* Calcd for $C_{12}H_{23}N_3O$: C, 63.96; H, 10.29; N, 18.65. Found: C, 63.23; H, 10.17; N, 18.29.

3-methyl-1-(2-(pyrrolidin-1-yl)ethyl)-1H-pyrazol-5(4H)-one (RA6): Yield 59%; mp 118-119 °C; IR (KBr) *v* cm^{-1}: 2995, 1701, 1516, 1508, 1251. ^{1}H-NMR (DMSO-*d6*) δ: 9.08 (s, -NH, 1H), 5.50 (s, -CH, 1H), 4.42 (t, -CH_2, 2H), 3.63 (t, -CH_2, 2H), 2.41 (t, -CH_2,

2H), 2.23 (s, -CH_3, 3H), 2.12 (m, -CH_2, 2H). MS *m/z*: 196 (M+1). *Anal.* Calcd for $C_{10}H_{17}N_3O$: C, 61.51; H, 8.78; N, 21.52. Found: C, 61.31; H, 8.43; N, 21.14.

3-methyl-1-(2-(piperidin-1-yl)ethyl)-1H-pyrazol-5(4H)-one (RA7): Yield 69%; mp 129-130 °C; IR (KBr) *v* cm^{-1}: 2999, 1704, 1519, 1507, 1258. ^{1}H-NMR (DMSO-*d6*) δ: 9.08 (s, -NH, 1H), 5.50 (s, -CH, 1H), 4.42 (t, -CH_2, 2H), 3.63 (t, -CH_2, 2H), 2.43 (t, -CH_2, 2H), 2.23 (s, -CH_3, 3H), 2.14 (m, -CH_2, 2H). MS *m/z*: 210 (M+1). *Anal.* Calcd for $C_{11}H_{19}N_3O$: C, 63.13; H, 9.15; N, 20.08. Found: C, 63.36; H, 9.32; N, 20.46.

3-methyl-1-(2-morpholinoethyl)-1H-pyrazol-5(4H)-one (RA8): Yield 65%; mp 111-112 °C; IR (KBr) *v* cm^{-1}: 2988, 1700, 1517, 1509, 1261. ^{1}H-NMR (DMSO-*d6*) δ: 9.08 (s, -NH, 1H), 5.50 (s, -CH, 1H), 4.42 (t, -CH_2, 2H), 3.63 (t, -CH_2, 2H), 2.23 (s, -CH_3, 3H), 2.63 (-CH_2, 2H), 4.10 (t, -CH_2, 2H). MS *m/z*: 212 (M+1). *Anal.* Calcd for $C_{10}H_{17}N_3O$: C, 56.85; H, 8.11; N, 19.89. Found: C, 56.63; H, 8.12; N, 19.34.

3-methyl-1-(2-(4-methylpiperazin-1-yl)ethyl)-1H-pyrazol-5(4H)-one (RA9): Yield 68%; mp 134-135 °C; IR (KBr) *v* cm^{-1}: 2995, 1709, 1515, 1505, 1268. ^{1}H-NMR (DMSO-*d6*) δ: 9.08 (s, -NH, 1H), 5.50 (s, -CH, 1H), 4.42 (t, -CH_2, 2H), 3.63 (t, -CH_2, 2H), 3.14 (s, -CH_3, 3H), 2.43 (t, -CH_2, 2H), 2.23 (s, -CH_3, 3H). MS *m/z*: 225 (M+1). *Anal.* Calcd for $C_{11}H_{20}N_4O$: C, 58.90; H, 8.99; N, 24.98. Found: C, 58.36; H, 8.23; N, 24.14.

4. Biological Activities

4.1. *Antitumor Activity*

In vitro anticancer activity of compounds **RA1-RA9** against EAC cells was determined by trypan blue exclusion method [16]. The EAC cells were collected, counted and adjusted to 106 cells/mL with normal saline. The drug dilutions were made with phosphate buffer saline (PBS) and were further adjusted to concentrations ranging from 125-1000 µg/mL. The drug dilutions were then added to the EAC cells and incubated at 37 °C for 3 h. At the end of 3 h, the cell viability was determined and percentage cytotoxicity was calculated. The percentage cytotoxicity was calculated using the formula:

$$\text{Percentage cytotoxicity} = 100 - T_c - D_c/T_c \times 100$$

where, T_c = total EAC cells and D_c = dead EAC cells [17].

Compounds **RA1-RA9** with significant *in vitro* anticancer activity were further selected for screening *in vivo* anticancer activity by determining different parameters like body weight analysis, mean survival time (MST) and percentage increase in life span (% ILS) [18]. The EAC cells containing 106 cells/0.1 mL of phosphate buffer saline were injected into the peritoneal cavity of all the animals (six swiss albino mice in each group). Treatment with test compounds (90 mg/kg body weight) and the standard 5-flurouracil

(520 µg/kg body weight) was started 24 h after inoculation of cancer cells, once daily as a single dose in 0.3% CMC suspension by intraperitoneal route for 10 days. All the mice were weighed daily up to 11 days. The decrease in body weight and MST (Mean Survival Time) of the test and standard group animals were compared with control group. Results are shown in Table 1. Percentage decrease in the body weight was determined by using the formula, percentage decrease in body weight = $(G_c - G_t) / G_c \times 100$, where G_c = gain in body weight of control group and G_t = gain in body weight of treated group. Percentage increase in life span was calculated by the formula, % ILS = (MST of treated group - MST of control group) / MST of control group × 100. Student t-test was performed to ascertain the significance of the exhibited activity.

Table 1. In-vitro and in-vivo anticancer activity of compounds (RA1-RA9).

Compounds	In-vitro anticancer activity % cyto-toxicity of drugs at various conc. on EAC cells (µg/ml)				In-vivo anticancer activity			
	900	600	300	150	Gain in body weight	% decrease in body wt.	MST[a)]±S.E.[b)]	% ILS[c)]
Control	-	-	-	-	5.01±0.02	-	14.10±0.23	-
RA1	87.0	79.0	56.1	49.2	0.88±0.028	82.43	34.50±0.19	144.68**
RA2	82.7	72.8	41.9	39.7	0.94±0.023	81.23	30.85±0.24	112.76**
RA3	79.1	70.8	40.1	37.3	1.03±0.064	79.44	29.18±0.25	106.95**
RA4	86.1	74.4	53.0	42.1	0.84±0.023	83.23	35.80±0.18	153.90**
RA5	81.5	72.6	41.9	39.8	0.95±0.14	81.03	29.86±0.29	111.77**
RA6	26.1	22.4	15.0	11.8	2.30±0.32	54.09	17.53±0.24	24.32*
RA7	16.7	13.6	11.3	9.1	4.15±0.11	17.16	15.70±0.05	11.34**
RA8	19.1	18.7	12.8	10.1	3.17±0.14	36.72	16.49±0.38	16.95*
RA9	89.2	73.5	53.0	47.1	0.83±0.010	99.40	37.50±0.19	165.95**
5-flurouracil	83.1	72.3	43.2	40.1	0.91±0.013	81.83	32.15±0.25	128.01**

[a)] MST = mean survival time, [b)] S.E. = standard error, [c)] %ILS = percentage increase in life span,
** $P < 0.01$ * $P < 0.05$.

Hematological analysis [17]

In order to detect the influence of **RA1-RA9** on hematological status of EAC-bearing mice, a comparison was made among four groups ($n = 6$) of mice on the 13th day after tumor inoculation. Group I comprised of normal mice, group II comprised of EAC bearing mice, group III comprising EAC bearing mice treated with (RA1-RA9) (90 mg/kg/day for 10 days), and group IV having EAC bearing mice treated with 5-flurouracil (520 µg/kg for 10 days). Blood was drawn from each mouse by the retro orbital plexus method and the white blood cell count (WBC), red blood cell count (RBC), hemoglobin and percentage differential count were determined. The results are given in Table 2.

Table 2. Determination of hematological parameters of (RA1-RA9).

Compounds	Hb (g %)	RBC (million/mm^3)	WBC (10^3 cells/mm^3)	Differential count %		
				Lymphocytes	Neutrophils	Monocytes
RA1	13.5± 0.07	4.2 ± 0.06	13.6 ± 0.08	74.3 ± 0.19	31.1 ± 0.11	1 ± 0
RA2	12.7 ± 0.07	3.8 ± 0.08	14.5 ± 0.12	72.2 ± 0.19	28.1 ± 0.11	1 ± 0
RA3	12.5 ± 0.10	3.7 ± 0.06	14.8 ± 0.08	71.3 ± 0.18	27.1 ± 0.16	2 ± 0
RA4	13.4 ± 0.41	4.1 ± 0.10	13.6 ± 0.07	74.5 ± 0.11	30.0 ± 0.07	1 ± 0
RA5	12.8 ± 0.09	3.8 ± 0.06	15.3 ± 0.12	73.2 ± 0.38	28.1 ± 0.12	1 ± 0
RA6	7.0 ± 0.13	2.4 ± 0.10	19.3 ± 0.08	29.1 ± 0.12	71.1 ± 0.19	2 ± 0
RA7	6.2 ± 0.07	2.8 ± 0.08	18.0 ± 0.19	31.3 ± 0.19	69.2 ± 0.28	2 ± 0
RA8	6.9 ± 0.10	3.0 ± 0.08	21.6 ± 0.73	25.4 ± 0.30	73.5 ± 0.44	2 ± 0
RA9	13.6 ± 0.05	4.2 ± 0.08	13.3 ± 0.08	74.5 ± 0.26	30.0 ± 0.05	1 ± 0
5-flurouracil	13.3 ± 0.10	4.1 ± 0.09	13.8 ± 0.12	73.2 ± 0.28	31.0 ± 0.08	1 ± 0
I	14.2 ± 0.31	4.5 ± 0.06	7.0 ± 0.08	75.1 ± 0.11	29.1 ± 0.12	1 ± 0
II	7.2 ± 0.12	2.2 ± 0.09	22.1 ± 0.11	23.5 ± 0.29	74.1 ± 0.10	2 ± 0

I = group comprising normal mice, II = group having EAC bearing mice.

4.2. *Antibacterial and antifungal activities*

Applying the agar plate diffusion technique [19] all of the newly synthesized compounds were screened *in vitro* for antibacterial activity against *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (Gram-negative), *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive) at 20 μg/ml, 30 μg/ml, 40 μg/ml concentrations, respectively. Under identical conditions, the positive control antibiotics Amoxicillin at 40 μg/ml showed zone of inhibition 26-24 mm and 22-25 mm for Gram-negative and Gram-positive organism respectively. Similarly, the antifungal screening of the compounds was carried out against fungi *C. albicans* and *A. niger* by using fluconazole (40 mg/ml) as the positive control, which showed 27 mm and 28 mm, respectively, as the zone of inhibition.

5. Results and Discussion

In vivo antitumor screening reveals that some of the tested compounds are promising candidates having good activity against EAC cells. Compounds **RA1, RA4**, and **RA9** exhibit highest antitumor activity than standard antitumor agent. Compounds **RA2, RA3, RA5** exhibit nearly same antitumor activity comparable to standard. Compounds **RA6, RA7, RA8** are inactive against EAC cells. Antimicrobial study reveals that compounds **RA1, RA4, RA6** and **RA9** exhibit good antibacterial and antifungal activities (Tables 3 and 4). Compound **RA9** exhibited the highest degree of antimicrobial activity than standard drugs. Rest of the compounds does not show any significant antimicrobial activity as compared with standard agent.

Table 3. Antibacterial Activity of Compounds[a] (**RA1-RA9**).

Comp.	*Escherichia coli*			*Pseudomonas aeruginosa*			*Bacillus subtilis*			*Staphylococcus aureus*		
	20 (µg/ml)	30 (µg/ml)	40 (µg/ml)	20 (µg/ml)	30 (µg/ml)	40 (µg/ml)	20 (µg/ml)	30 (µg/ml)	40 (µg/ml)	20 (µg/ml)	30 (µg/ml)	40 (µg/ml)
RA1	11	18	23	13	18	23	13	20	24	12	19	24
RA2	-	-	-	4	6	9	3	7	11	5	-	8
RA3	3	7	10	2	5	9	4	7	10	4	7	11
RA4	10	15	22	12	20	23	11	17	23	12	18	22
RA5	4	7	10	3	7	11	3	6	12	4	6	12
RA6	10	16	22	11	14	21	10	17	22	12	18	22
RA7	5	9	12	-	-	-	4	8	13	4	7	9
RA8	6	10	15	5	7	11	6	9	13	6	8	10
RA9	13	23	28	14	22	26	14	21	28	14	22	26
Amoxicillin	12	20	26	-	-	24	14	22	27	12	-	25

[a] = Zone of inhibition in millimeter.

Table 4. Antifungal Activity of Compounds[a] (**RA1-RA9**).

Compounds	*Candida albicans*			*Aspergillus niger*		
	20 (µ/ml)	30 (µ/ml)	40 (µ/ml)	20 (µ/ml)	30 (µ/ml)	40 (µ/ml)
RA1	12	18	24	11	20	25
RA2	5	7	14	3	-	-
RA3	3	6	10	2	4	5
RA4	11	19	24	14	21	27
RA5	4	7	10	5	9	17
RA6	13	18	22	11	20	24
RA7	5	6	12	3	7	11
RA8	4	6	9	1	4	6
RA9	15	22	27	13	22	29
Amoxicillin	14	23	27	-	-	28

[a] = Zone of inhibition in millimeter.

Structure activity studies of the title compounds for antitumor activity reveal the importance of N-1 position of pyrazolone ring as it should contain N-methyl substitution followed by ethyl linkage as **RA1** (144.68), **RA4** (153.90), **RA9** (165.95) increases the percentage increases in life span (%ILS) as compared to compound **RA6** (24.32), **RA7**

(11.34), **RA8** (16.95) having heterocyclic substitution at N-1 position followed by ethyl linkage.

Hematological parameters of EAC bearing mice on day 13 showed significant changes when compared to normal mice (Table 2). The total WBC count was found to increase with a reduction in the hemoglobin content of RBC. The differential count of WBC showed that the percentage of neutrophils increased while that of lymphocytes decreased. At the same time treatment with compounds **RA1**, **RA4**, and **RA9** could change these altered parameters to near normal.

Acknowledgements

We gratefully acknowledge the UGC Networking Centre, School of Chemistry, University of Hyderabad, for providing laboratory facilities for synthesis and Spectral Analysis. IISc (Bangalore) for the Spectral analysis. Also we are thankful to the Divakar Goli, Principal, Acharya & B.M. Reddy College of Pharmacy for providing laboratory facilities.

References

1. C. Daniele, D. L. Alessandro, R. Marco, B. Beatrice, M. Fabrizio, and M. Matteo, Bioorg. Med. Chem. **16,** 8587 (2008). http://dx.doi.org/10.1016/j.bmc.2008.08.016
2. R. V. Ragavan, V. Vijayakumar, and N. S. Kumari, Eur. J. Med. Chem. **44,** 3852 (2009). http://dx.doi.org/10.1016/j.ejmech.2009.04.010
3. M. F. Brana, A. Gradillas, A. G. Ovalles, B. Lopez, N. Acero, and F. Llinares, Bioorg. Med. Chem. **14,** 9 (2006). http://dx.doi.org/10.1016/j.bmc.2005.09.059
4. K. R. Kim, J. L. Kwon, J. S. Kim, N. Zaesung, H. R. Kim, and H. G. Cheon, Eur. J. Pharmacol. **528,** 37 (2005). http://dx.doi.org/10.1016/j.ejphar.2005.10.027
5. X. Fan, X. Zhang, L. Zhou, K. A. Keith, E. R. Kernb, and P. F. Torrencea, Bioorg. Med. Chem. Lett. **16,** 3224 (2006). http://dx.doi.org/10.1016/j.bmcl.2006.03.043
6. V. C. Filho, R. Correa, Z. Vaz, J. B. Calixto, R. J. Nunes, T. R. Pinheiro, A. D. Andricopulo, and R. A.Yunes, Farmaco. **53,** 55 (1998). doi:10.1016/S0014-827X(97)00006-2
7. S. M. Sondhi, Sharma, R. P. Verma, N. Singhal, R. Shukla, R. Raghubir, and M. P. Dubey, Synthesis.878 (1999). http://dx.doi.org/10.1055/s-1999-3472
8. M. M. F. Ismail, Y. A. Ammar, H. S. A. El-Zahaby, S. I. Eisa, and S. E. Barakat, Arch. Pharm. Life. Sci. **340,** 476 (2007).
9. A. M. El-Hawash, A. M. B. El-Sayed, and I. M. El-Ashmawey, Eur. J. Med. Chem. **41,** 155 (2006). http://dx.doi.org/10.1016/j.ejmech.2005.09.006
10. F. R. Souza, V. T. Souza, V. Ratzlaff, L. P. Borges, M. R. Oliveira, H. G. Bonacorso, N. Zanatta, M. A. P. Martins, and C. F. Mello, Eur. J. Pharmacol. **451,** 141 (2002). http://dx.doi.org/10.1016/S0014-2999(02)02225-2
11. A. Mohd and S. Kumar, Indian J. Chem. **44** B, 2532 (2005).
12. M. P. Clark, S. K. Laughlin, M. J. Laufersweiler, R. G. Bookland, T. A. Brugel, A. Golebiowski, and M. P. Sabat, J. Med. Chem. **47,** 2724 (2004). http://dx.doi.org/10.1021/jm049968m
13. S. Kuppusamy, S. Gnanamani, P. S. Nagarajan, M. Seeralan, T. P. Paramasivan, and R. Melani, Bioorg. Med. Chem. Lett. **19,** 4501 (2009). http://dx.doi.org/10.1016/j.bmcl.2009.02.113
14. P. Camilleri, D. P. Astles, M. W. Kerr and J. E. Spencer, J. Agric. Food. Chem. **38**, 1601 (1990). http://dx.doi.org/10.1021/jf00097a036
15. A. Kimata, H. Nakagawa, R. Ohyama, T. Fukuuchi, S. Ohta, T. Suzuki, and N. Miyata, J. Med. Chem. **50,** 5053 (2007). http://dx.doi.org/10.1021/jm070688r

16. V. Murugan, M. Kulkarni, R. M. Anand, E. P. Kumar, B. Suresh, and V. M. Reddy, Asian J. Chem. **18,** 900 (2006).
17. N. M. Raghavendra, P. M. Gurubasavarajaswamy, K. S. Nagaranavile, and T. Parameshwaran, Arch. Pharm. Res. **32** (3), 431 (2009). http://dx.doi.org/10.1007/s12272-009-1317-8
18. J. A. Khanam, S. P. Bag, B. Sur, and P. Sur, Ind. J. Pharmacol. **29,** 157 (1997).
19. V. Mathew, J. Keshavayya, V. P. Vaidya, and D. Giles, Eur. J. Med. Chem. **42**, 823 (2007). http://dx.doi.org/10.1016/j.ejmech.2006.12.010
20. C. Sharma, B. Thadhaney, G. Pemawat, and G. L. Talesara, Indian J. Chem. **47** B, 1892 (2008).